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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/031,410	06/21/2002	Peter Eriksson	59760 (47137)	2145
21874	7590	05/03/2007		
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			ART UNIT	PAPER NUMBER
			1636	
			MAIL DATE	DELIVERY MODE
			05/03/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary

Application No.

10/031,410

Applicant(s)

ERIKSSON ET AL.

Examiner

Laura McGillem

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 01 February 2007.
- 2a) ☐ This action is FINAL. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-6, 8-22, 25-29, 31-33 and 35 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-6, 8-22, 25-29, 31-33 and 35 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☒ All b) ☐ Some * c) ☐ None of:
1. ☒ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 2/1/2007 has been entered.

It is noted that claim 1 has been amended and claim 35 has been added in the amendment filed 2/1/2007. Claims 1-6, 8-22, 25-29, 31-33 and 35 are under examination.

Oath/Declaration

Applicant's arguments, see REMARKS (page 1), filed 2/1/2007, with respect to the oath have been fully considered and are persuasive. The objection of the oath has been withdrawn.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 29 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant

regards as the invention. Claim 29 is vague and indefinite because it is drawn to treatment of a tumor using the method of claim 1, but claim 1 is an *in vitro* method. As the claim is written, it does not comprise limitations regarding how *in vitro* electrofused cell will be used to treat a tumor.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 29 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a NEW MATTER rejection.

Claim 29 is a method for treatment of a tumor comprising using the method according to claim 1, but claim 1 has been amended to limit the method to an *in vitro* method. Therefore in order to use the method of claim 29 comprising using an *in vitro* method, the skilled artisan would have to practice the method of electrofusion of two fusion partners having cell-like membranes *in vitro* and then use the electrofused partners to treat a tumor which encompasses an *ex vivo* treatment method. The instant specification does not disclose or contemplate such a method for tumor treatment. The instant specification discloses methods of *in vitro* fertilization, which would encompass *in vitro* fusion of an egg cell and a sperm cell for implantation (e.g. *ex vivo* procedure).

However, the specification only discloses tumor treatment in the context of an *in vivo* electrofusion method (see page 11-12 and Figure 5). Therefore, the amendment to claim 1 changes the method of claim 29 so that claim 29 is now drawn to an *ex vivo* tumor treatment method and constitutes impermissible new matter.

Claims 1-6, 8-22, 25-28, 31-33 and 35 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for *in vitro* selective electrofusion of at least two fusion partners having cell-like membranes, does not reasonably provide enablement for conducting *in vitro* fertilization by selective electrofusion of an egg cell or an enucleated egg cell, and a sperm cell at any development stage, or for conducting non-human cloning. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims. Claim 35 is newly added to this rejection.

It is noted that claim 1 has been amended to limit the method to *in vitro* embodiments. Furthermore, new claim 35 recites the limitation that the "*in vitro* method is an *ex vivo* procedure" which encompasses *in vitro* fertilization methods followed by implantation into animals and methods of non-human cloning.

This rejection is being maintained for reasons of record in the previous Office Action, mailed 2/1/2007 and for reasons outlined below. Applicants point out that, according to the invention as currently claimed, it would not require a great deal of work to perform an *in vitro* method for selective electrofusion of at least two fusion

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partners having cell-like membranes. Applicants submit that the specification provides teaching of electrical field strength and number (p.8), strength and duration of fusion pulse (p.8), positioning of the electrodes (p.9), and preferred dimension of the electrodes (p.9). Applicants submit that the specification teaches preparation of fluorescence encapsulated vesicles for use in the method (p. 16), and chemicals and materials needed to perform the method (p. 17 - 18). Applicants submit that the specification provides examples of experimental setup and instrumentation (p. 14 - 15). The specification provides working examples of both cell-cell and cell-liposome fusion (p. 18 - 21).

Applicants cite *In re Wands* and submit that the experimentation to perform the claimed methods is merely routine and not undue. Applicants submit that the instant specification provides ample guidance to perform an *in vitro* method for selective electrofusion of at least two fusion partners having cell-like membranes. Applicants submit that any experimentation required to perform the method using, for example, any two fusion partners having cell-like membranes in an *in vitro* environment would not require more than routine experimentation.

Applicant's arguments filed 2/1/2007 have been fully considered but they are not persuasive.

The scope of the claims encompasses methods in which eggs or enucleated egg cells and sperm cells are fused, which is *in vitro* fertilization. The claims also encompass *in vitro* methods of selective electrofusion of at least two fusion partners having cell-like membranes using an electric field of a strength sufficient to obtain fusion

provided by at least one microelectrode that is sufficiently small to permit selective fusion in an *ex vivo* procedure. An *in vitro* fusion method in an *ex vivo* method encompasses *in vitro* fusion of egg cells, enucleated egg cells, and sperm cells at any development stage and then implanting them in a prepared animal. Therefore the scope of the claimed method encompasses a very large genus of two fusion partners such as egg cells, enucleated egg cells, and sperm cells from any animal species. The claims encompass cloning, excluding human cloning, of any other species besides humans. The specification does not disclose or contemplate the use of egg cells, enucleated egg cells or sperm cells at any development stage for any use other than *in vitro* fertilization or non-human cloning.

State of the Art and Unpredictability of the art As discussed in a previous Office Actions (4/13/2006) Sakai et al (of record) teaches that several mammalian species have been cloned by somatic cell nuclear transfer, but that the short and long term effects of cloning and assisted reproductive techniques are largely unknown (see page 152, left column, 1st paragraph). Sakai et al teach that systematic studies of cloned offspring are necessary, but are complicated by the low proportion of live offspring from nuclear transfer eggs, which is currently 2-3% regardless of species or nuclear transfer technique (see page 152, center column, 1st full paragraph). Sakai et al teach that studies of cloning and cloned animals are limited by the ability to generate a sufficient number of age-matched cloned animals and difficulty in designating appropriate controls (see page 152, right column, 1st paragraph). Sakai et al teach that reasons for low efficiency of cloning are currently unclear, but that exposure of eggs and

embryos to *in vitro* culture conditions, such as culture medium that may contain chemicals at non-physiological concentrations, can affect embryonic development and result in offspring abnormalities (see page 159, right column, 1st full paragraph, for example). Sakai et al teach that although *in vitro* fertilization techniques are widely used in livestock production, *in vitro* embryo culture has been associated with abnormal physiology and morphological development and a perinatal mortality at a higher rate than natural fertilization.

Niemann and Rath (of record) teach that there are differences in the progress of *in vitro* reproductive techniques among livestock such as cattle, sheep and swine. Sakai et al suggests that the amount of cellular trauma and damage to eggs, sperm and embryos during manipulation could result in negatively impacted cellular development and may depend on the technical skill of the individual manipulating the gametes and embryos (see page 160, left column, 1st paragraph, for example). Niemann and Rath also teach that success rates for *in vitro* fertilization of porcine embryos is much lower than that of cattle. Niemann and Rath teach that main problems for *in vitro* fertilization for swine include insufficient cytoplasmic maturation of oocytes, low proportion of blastocysts and high proportion of polyspermic fertilization (see abstract).

The unpredictability of using methods of *in vitro* fertilization and cloning is manifested in multiple issues. Sakai et al teach that cloning methods and studies of cloning outcomes are hampered by the very low rate of clones produced and the difficulty of establishing appropriate controls. Sakai et al preliminarily conclude that clones are not always phenotypically identical to the somatic cell donors and that the

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cloned progeny often have adverse health conditions, such as increased body weight and advanced aging. Sakai et al teach that cloning technique is "still unpredictable" and requires comprehensive and systematic longitudinal studies of cloned animals (see page 152, right column, 1st paragraph). Sakai et al disclose that pre- and perinatal death rates in clones are significantly higher than controls regardless of species and that the reason for low efficiency of somatic cell cloning are currently unclear (see page 153, center column, 1st paragraph). Sakai et al teach that the variety of findings in cloned animals of multiple species suggests that cloning has different consequences among different species and even within a species.

Amount of guidance provided. While Applicants submit that the instant specification provides sufficient guidance, the specification has not provided any guidance regarding the specific size and number of electrodes to be used for non-human cloning or *in vitro* fertilization. Applicants have not provided any guidance regarding the specific number, strength and duration of fusion pulses to be used for non-human cloning or *in vitro* fertilization. Applicants have not provided any guidance regarding the electrical field to be used for non-human cloning or *in vitro* fertilization. Applicants have not provided any guidance regarding differences in the claimed fusion method for any other cells beside those exemplified established cell lines, regarding potential variations related to cell type, age and growth conditions, especially for non-human cloning and *in vitro* fertilization. Specific to claim 35, Applicants have not provided any guidance regarding how cells subjected to a selective electrofusion

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methods will be returned to an animal in an *ex vivo* procedure especially for *in vitro* fertilization.

Working examples. Applicants have provided an example of cell-cell fusion of PC12 cells *in vitro*, Applicants disclose but do not show: fusion of NG108 cells in a network, fusion of NG108 cells, Jurkat cells and COS7 cells, and fusion of NG108 cells to PC12 cell to create hybrid cells. Applicants do not provide information regarding specific number, strength and duration of fusion pulses for a cell-single vesicle fusion. Applicants have not provided any example of non-human cloning, *in vitro* fertilization of any organism, or an *in vitro* method comprising an *ex vivo* procedure.

Nature of the invention. The nature of the invention is drawn to *in vitro* cell electrofusion, *in vitro* fertilization and cloning of non-human organisms, which are very complex and controversial aspects of science and medicine to date.

Level of skill in the art. The level of skill in the art is high but due to the scope of the claimed method, the state and unpredictability of the art, lack of guidance and working example and nature of the invention, the skilled artisan would not know how to use the claimed method without practicing undue and excessive trial and error experimentation. Although Applicants submit that the any experimentation required to perform the method using any two fusion partners having cell-like membranes in an *in vitro* environment would not require more than routine experimentation, based on the *In re Wands* analysis of the Forman factors, the specification has not provided sufficient information so that the skilled artisan would know how to use the claimed method without undue and excessive experimentation.

As *In re Gardner, Roe and Willey*, 427 F.2d 786,789 (C.C.P.A. 1970), the skilled artisan might eventually find out how to use the invention after "a great deal of work". In the case of *In re Gardner, Roe and Willey*, the invention was a compound which the inventor claimed to have antidepressant activity, but was not enabled because the inventor failed to disclose how to use the invention based on insufficient disclosure of effective drug dosage. The court held that "the law requires that the disclosure in the application shall inform them how to use, not how to find out how to use for themselves".

Claim 29 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the application coupled with information known in the art without undue experimentation *United States v. Telectronics, Inc.*, 8 USPQ2d 1217 (Fed. Cir. 1988). Whether undue experimentation is required is not based upon a single factor, but rather is a conclusion reached by weighing many factors. These factors were outlined in *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Inter. 1986) and again in *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988) and include the following:

1) Scope of the claims. Claim 29 is drawn to an *in vitro* method of selective electrofusion of at least two fusion partners having cell-like membranes for treatment of a tumor, which encompasses electrofusion of two fusion partners followed by administration to a tumor *in vivo*. The claims encompass a large genus of any type of tumor and any type of treatment that can be performed using a method of selective electrofusion of at least two fusion partners. The specification contemplates delivery of tumor treating genes known as suicide genes (cytokine deaminase or thymidine kinase). The instant claim appears to encompass a method in which the suicide gene would be introduced into cells which would then be administered to a tumor *in vivo*. It appears that the tumor cells adjacent to those that were electrofused would be treated by the bystander effect of suicide gene therapy (discussed below).

2) State of the Art. In a review published after filing of the instant application, Prasad et al (Curr. Med. Chem. 2004, Vol. 4 pages 347-361) suggest that methods of suicide gene therapy may be useful for treatment of gliomas. Prasad et al teach that in this process, a gene that is capable of converting a pro-drug into a toxic metabolite is introduced into tumor cells. For example, Prasad et al teach that HSV-tk will phosphorylate the antiviral agent ganciclovir (GCV), which causes arrest of DNA synthesis in tumor cells (see page 353, right column, 4th and 5th paragraph and Figure 5, for example). Prasad et al teach that the therapeutic value is enhanced by the "bystander effect" that occurs when the transfected tumor cells communicate the signal for apoptosis to neighboring cells. Prasad et al teach that several preliminary clinical trials have been conducted and although no significant adverse effects have been

observed, antitumor effects are limited. Prasad et al also teach a similar strategy using a microbial enzyme known as cytosine deaminase (CD), which converts an anti-fungal agent to a toxic metabolite. Prasad et al teach that tumor regression occurred in mice with intracerebral xenografts transfected with the cytosine deaminase (see page 354, left column, for example). Prasad et al summarize that approaches using HSV-TK and CD have a relatively robust bystander effects and potentially overcome some limitations of gene therapy. Prasad et al conclude that overall, more comprehensive patient trials are required before substantive assessment of these types of strategies can be made (see page 358, left column, for example).

Niculescu-Duvaz et al (Mol. Biotechnol. 2005, Vol. 30, No.1, pages 71-88) also teach methods known as suicide gene therapy or gene-directed enzyme pro-drug therapy (GDEPT). Niculescu-Duvaz et al teach that the gene for the enzyme should ideally be expressed exclusively in the tumor cells as compared to normal tissues and must reach a concentration sufficient to activate the pro-drug for clinical benefit. Niculescu-Duvaz et al also teach that the catalytic activity of the expressed protein must be adequate to activate the pro-drug under physiological conditions. Niculescu-Duvaz et al teach that because expression of the foreign enzyme will not occur in all cells of the targeted tumor *in vivo*, the bystander effect is required to kill neighboring cells.

3) Unpredictability of the art. The unpredictability of being able to use an *in vitro* electrofusion method in a tumor treatment is manifested in the possible side effects of the suicide gene system and the efficacy of the bystander effect. While Niculescu-Duvaz et al teach some advantages of the suicide gene therapy systems, Niculescu-

Duvaz et al also teach that control of gene expression in the tumor is a hurdle to be overcome (see page 72, for example). Further, some of the suicide genes encoding enzymes of non-mammalian origin either with or without human homologs are likely to be immunogenic (see page 72, left column, for example). Niculescu-Duvaz et al teach that there are other genes encoding enzymes that can be used for suicide gene therapy that would be less immunogenic, but since they would be present in normal tissues, specific activation of the prodrugs in tumors would likely not be possible (see page 73, left column). Niculescu-Duvaz et al teach that the pro-drugs should be stable under physiological conditions and also highly diffusible in the tumor interstitium. Further, the released drugs should be potent, capable of inducing the bystander effect (see page 73, left column, for example).

Niculescu-Duvaz et al teach that the HSV-TK/GCV system requires cell to cell contact to produce a bystander effect (see page 82, right column, for example). Niculescu-Duvaz et al teach that there have been a number of clinical trials with different suicide gene therapies and the side effects of the different components such as the enzymes or the prodrugs are an important consideration (see page 84, right column). Niculescu-Duvaz et al teach that there are some hurdles to be overcome before GDEPT can become a clinically efficient treatment of cancer. Niculescu-Duvaz et al suggest that enhancement and control of the bystander effect may be useful to improve the therapies. Improvements can also be made to produce more efficient pro-drug activation (see page 84, right column, for example).

4) Amount of guidance provided. The specification has provided some guidance regarding the specific number, strength and duration of fusion pulses required to fuse several well-known cell types *in vitro*. The specification has provided some guidance regarding the dimensions of a specific hollow electrode used to fuse two cells *in vitro*. The specification discloses that for methods using multiple pulses a repetition rate of ~1 Hz should be suitable. The specification discloses that the length and strength of the pulses depend on the size of the partners to be fused. The specification provides preferable size ranges for the microelectrodes.

The Applicants have not provided any guidance regarding the specific size and number of electrodes to be used, or the specific number, strength and duration of fusion pulses for *in vitro* electrofusion in a method to treat a tumor. The specification does not provide information regarding how electrofused cells will be used to treat a tumor (i.e. administered systemically, locally, etc). The specification does not provide information regarding how many electrofused cells would be needed to treat a tumor or how many times they would need to be administered. Applicants have not provided any information concerning any variations in the claimed method for various pharmaceutical agents to be delivered to cells or how the composition of the cell membrane would be altered for treatment of tumors. If the disclosed suicide gene system is to be used, Applicant have not provided guidance regarding activation of the enzymes or efficacy of the bystander effect that appears to be necessary to treat tumor cells adjacent to cells that have been electrofused *in vitro*. Therefore the Applicants have not provided sufficient guidance so

that the skilled artisan would be able to perform the claimed method without undue trial and error experimentation.

5) Working examples. Applicants have provided an example of cell-cell fusion of PC12 cells *in vitro*. Applicants disclose fusion of NG108 cells in a network, fusion of NG108 cells, Jurkat cells and COS7 cells, and fusion to create hybrid cells. Applicants have provided an example of cell-single vesicle fusion and do not provide information regarding specific number, strength and duration of fusion pulses. Applicants have provided a third example in which NG108 cells are fused using electrolyte-filled silica capillary electrodes which are defined by their dimensions and include information regarding specific number, strength and duration of fusion pulses required to fuse the cells. The specification does not provide any example of *in vitro* selective electrofusion of at least two fusion partners having cell-like membranes for a treatment of a tumor *in situ*. Applicants contemplate intracellular drug or gene administration *in vivo* into tumor using an electrofusion process (see pages 11-12) but do not provide an example.

6) Nature of the invention. The nature of the claimed methods is drawn to tumor treatment using methods of fusion partners having cell-like membranes to introduce therapeutic pharmaceuticals and administration to a tumor, which are complex and unpredictable aspects of science and medicine.

7) Level of skill in the art. The level of skill in the art is high but given the nature of the invention, the scope of the claimed method, the state and unpredictability of the art, lack of guidance and working examples, the skilled artisan would not know how to use the claimed method without excessive trial and error experimentation.

Given the above analysis of the factors which the Courts have determined are critical in ascertaining whether a claimed invention is enabled, it must be considered that the skilled artisan would have had to have practiced undue and excessive experimentation in order to practice the claimed invention.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claim 1 and 17 are rejected under 35 U.S.C. 102(b) as being anticipated by Magae et al (Appl. Micro. Biotechnol., 1986, Vol. 24, 509-511).

This rejection is being maintained for reasons of record in the previous Office Action, mailed 11/3/2006 and for reasons outlined below.

Applicants submit that the Examiner argues that "the disclosure does not provide a specific definition of the phrase 'highly focused' that would exclude the method taught by Magae et al. (Office Action, p. 10)." Applicants submit that the meaning of the term "highly focused" is well-supported and described in the specification. For example, on page 8, lines 30 - 37, the specification teaches:

The electrical field used in step B to obtain fusion should be *highly focused in order to avoid affecting any surrounding structures*...To focus the electrical field it is preferable to provide the electrical field by use of one or two microelectrodes positioned close to the two fusion partners, i.e. 0 - 10 μm , preferably 0 - 5 μm , from the cellular membrane. (emphasis added by Applicants).

Applicants submit that the term "highly focused" is made even clearer when read in light of the preceding paragraphs on page 8 that describe how the electrical field is obtained (lines 8 - 30), as well as the Examples. In particular, Example 3 (p.22) teaches positioning for cell fusion using single open-bore capillaries, and specifically positioning of the capillary tip using micromanipulators and fusion of two aligned cells by applying pulses of 5 to 15 kV for 0.1 - 5 seconds.

As the Examiner states, the Magae et al reference teaches, "the electrofusion of plant protoplasts occurs in a drop of protoplast solution on a cover glass and results in the fusion of 10 to 20 pairs of protoplasts (Office Action, p. 10)." Specifically, the Magae et al reference teaches on page 509 that, "fusion frequency was estimated as the number of fused-protoplasts that had achieved the final stage; that is a completely spherical form, *among 10 to 20 pairs of protoplasts* (emphasis added)." Applicants submit that contrary to the Examiner's assertion, the method of Magae et al does not meet the limitation of electrofusion of at least two fusion partners using a highly focused electric field, but rather teaches bulk fusion of structures in a solution. Applicants submit that the Magae et al reference fails to teach a method of fusion that is highly focused in order to avoid affecting any surrounding structures, but rather teaches a method of

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fusion whereby structures in a protoplast solution are induced to fuse in an un-focused manner. Applicants submit that Magae et al teach a method to fuse two giant plant protoplasts using glass electrodes attached to a micromanipulator. Applicants submit that the instant method is distinguished over the prior art in using at least a single electrode to provide a highly focused electric field for the fusion of at least two fusion partners. Applicants submit that the instant invention is based on a method that allows controllable fusion of single cells.. Applicants submit that the Magae et al do not provide a method of selective electrofusion that comprises bringing the fusion partners in to contact and applying a highly focused electric field. Applicants submit that the method of Magae et al is a bulk electrofusion, made more efficient by manipulation of the conditions and size of the cell.

Applicant's arguments filed 2/1/2007 have been fully considered but they are not persuasive. It appears that the issues regarding this rejection are based on the limitation of "highly focused" and "at least two fusion partners".

Applicants have submitted that the disclosure of "highly focused" as to "avoid affecting any surrounding structures" is a limiting definition of the phrase "highly focused". However, the phrase "in order to avoid affecting any surrounding structures", is not a limiting definition because it is a broad disclosure. The phrase "surrounding structures" is a broad term and could encompass not only any surrounding potential fusion partners but also, using the broadest reasonable interpretation, could encompass the surrounding structures of the microscope and micromanipulator equipment. One of skill in the art of electrophysiology knows that procedures employing electrical fields are

delicate and subject to outside electrical interference. Further, the word "affecting" is not limiting. The skilled artisan does not know the metes and bounds of how an electrical field would have to be "affecting" a surrounding structure in order to be considered not highly focused. Therefore, the electric field taught by Magae et al meets the limitation of an electric field of a strength sufficient to obtain fusion and highly focused on the fusion partners.

Regarding the limitation of at least two fusion partners, Magae et al teach fusion "among 10 to 20 pairs of protoplasts" apparently in the context of the number of fused protoplasts that have achieved a final stage in order to estimate fusion frequency (see page 509, right column, 3rd full paragraph, last 3 lines). Magae et al specifically recite that a "single electrical pulse was applied discharging a capacitor... to two protoplasts in contact between the microelectrode" (see page 509, right column, 3rd full paragraph, for example). Figure 1 on page 510 illustrates the fusion of only two protoplasts. It appears that Magae et al applied an electrical pulse to 10 to 20 pairs of protoplasts total (i.e. pulsing of multiple pairs) and not at the same time. Using the broadest reasonable interpretation of the limitation "at least two fusion partners" it appears that fusion of the two protoplasts as taught by Magae et al meet the limitation of the claimed method.

Claims 1- 2, 8-12, 15-19 and 26-29 are rejected under 35 U.S.C. 102(e) as being anticipated by (Pui et al) U.S. Patent No. 6,093,557, filed 6/5/1998.

This rejection is being maintained for reasons of record in the previous Office Action, mailed 11/3/2006 and for reasons outlined below.

Applicants submit that the teaching of the '557 reference does not anticipate the claimed method for selective electrofusion of at least two fusion partners having cell-like membranes using a highly focused electric field. Applicants submit that the Examiner argues, again, that "the disclosure does not provide a specific and limiting definition of the phrase 'highly focused', and the word 'selective' is not specifically defined in the disclosure (Office Action, p. 12)." Applicants refer to the arguments presented above, and maintain that the meaning of the term "highly focused" is well-supported and described in the specification, see page 8, lines 30 - 37.

Applicants submit that the '557 reference fails to teach a method of fusion that is highly focused but, as the Examiner points out on page 12 of the Office Action, teaches a "method that establishes a spray of substantially dispersed particles that have an electrical charge applied thereto (col. 3)." The Examiner argues that "the electrospraying method of [the '557 reference] is not for the purpose of impact penetration (but that) in addition to the penetration of cells as a result of bombardment, electrospraying techniques can be used to direct liposome droplets over the target cells...to facilitate transfer of material into the cell through fusion (Office Action, p. 12)." Applicants submit that the Examiner's argument does not relate to the claims at hand. In order to anticipate a claim, each and every element of the claim must be found in a single reference. Applicants cite the Manual of Patent Examining Procedure § 2131.

Applicants submit that the method of fusion taught by the '557 uses a spray that is established in the region of a target that includes one or more cells (see Figure 1A, description). Applicants submit that the spray of charged particles provides neither

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selectivity of fusion between the two partners, nor does it provide an electric field that is highly focused on the fusion partners. Applicants submit that the '557 reference contains no reference to a highly focused electric field as taught by the instant invention, nor does it mention selectivity of fusion between two fusion partners. Applicants submit that rather, the '557 reference teaches a method involving "a spray of substantially dispersed particles (claim 1)" wherein one or more of the substantially dispersed particles is introduced into the target cell.

Applicant's arguments filed 2/1/2007 have been fully considered but they are not persuasive. It appears that the issues surround these rejections are based in part on the limitation of "highly focused". The limitation of highly focused has been discussed in the above rejection. The specification does not provide a limiting definition of the phrase "selective electrofusion" and the Applicants have not pointed to disclosure that supports a limiting definition of the phrase "selective electrofusion".

Further, Pui et al teach that the spray can be confined to one or more target cells (see column 3, lines 14-20, column 5, lines 33-35, and column 6, lines 46-65, in particular). In column 14, lines 52-67. Pui et al teach:

In addition to penetration of the cells as a result of the bombardment of the cells with material using the present invention, the electrospraying technique described herein may be used to produce liposome droplets encapsulating biological material, e.g., DNA. The liposome droplets can be directed by the electric field and distributed uniformly over target cells in manners similar to those described herein, e.g., movement of the target surface, movement of the distributor head, etc. **As opposed to the penetration of the cells at impact, the liposomes incapsulating the biological material facilitate transfer of the material into the cells through fusion of the liposome with the cell membrane as is known to those skilled in the art.** The liposome droplets may be of varying sizes, e.g., a nominal diameter of about 10 nm to about 10 μm . The electrospraying technique used to direct the

liposomes onto the cells can be adjusted (e.g., distance of nozzle to target surface can be adjusted, **electrical potential or strength of the field can be adjusted, etc.) to vary the velocity of the liposome droplets such that the liposome droplets land appropriately for the fusion mechanism to be accomplished.** (Emphasis added by Examiner).

Therefore Pui et al do teach fusion of liposome droplets to cells using an electrospraying technique in which the electrical potential or field strength can be adjusted. Pui et al also teach that the spray of charged particles is confined or directed toward one or more target cells (see column 6, lines 26-31). Although Pui et al does not use the exact phrases "selective electrofusion" and "highly focused", using the broadest reasonable interpretation of the claimed method, a confined or directed spray of charged particles would be providing highly focused electric field for selective electrofusion of at least two fusion partners. Pui et al teach formation of non-uniform electric fields between electrodes (see column 9, lines 35-67, for example). The instant disclosure and the claims as written do not exclude an electrospraying technique as taught by Pui et al. Therefore, using the broadest reasonable interpretation, the teaching of Pui et al anticipate the claimed method.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Laura McGillem whose telephone number is (571) 272-8783. The examiner can normally be reached on M-F 8:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Laura McGillem, PhD
Examiner
4/24/2007

CELINE QIAN, PH.D.
PRIMARY EXAMINER

